Chapter-I: Introduction
1.1. Malaria

Malaria is a parasitic disease, caused by the protozoan parasite *Plasmodium*, and spread by the female mosquitoes of the genus *Anopheles*. Human malaria is caused by four parasite species *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. In addition a fifth malaria parasite species, *Plasmodium knowlesi*, though responsible for simian malaria, has shown to infect human populations as well (Chin et al., 1965; Cox-Singh et al., 2008). The most virulent of the human malaria parasites is *Plasmodium falciparum* responsible for the high rate of disease related morbidity and mortality (Perkins et al., 2011). *P. vivax* is less dangerous but more widespread, and the other three species are found much less frequently (World Malaria Report, 2011).

Malaria affects 106 endemic countries around the world (Figure 1). Estimates show that 216 million malaria cases have been reported in 2010 worldwide, with 655,000 malaria deaths, and Africa accounting for 81% of malaria cases followed by South East Asia at 13% (World Malaria Report, 2011). India alone accounts for 76% of the total malaria cases being reported in South East Asia (Kumar et al., 2007) and with 95% of the population residing in malaria endemic regions, each year 1.5 million people get affected from malaria and 1000 succumb to the disease (National Vector Borne Diseases Control Programme website). However, these official numbers could be far underestimated as reported in the national survey conducted by Dhingra et al., 2010. According to their data a whopping 0.2 million malaria deaths have been estimated per year, which is enormously high from the official estimates (Dhingra et al., 2010). In India, three *Plasmodium* species are prevalent *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* (Kumar et al., 2007). Recent estimates have revealed that even though there is a decline in the incidence of malaria infection caused by *Plasmodium falciparum* since 1995 from 1.14 million to 0.77 million per year. In 2010 the percentage of *Plasmodium falciparum* malaria cases
has increased from 39% to 52% compared to the other commonly existing *Plasmodium* species in India, *Plasmodium vivax* and *Plasmodium malariae* (NVBDCP website).

**Figure-1** Global Malaria Distribution, (World Malaria Report, 2011, World Health Organization)

**1.2. History of Malaria**

Malaria is probably one of the oldest diseases with its mention in the ancient Roman and Chinese manuscripts, more than 4000 years ago. The term malaria originated when Italians referred to intermittent fever as mal’aria (mala – bad, aria – air) caused by exposure to the marshes.

The first breakthrough discovery in malaria was made by a French physician, Charles Louis Alphonse Laveran in 1888, when he identified the malaria parasite, and named it as *Oscillatoria malariae*, which later was given the name of *Plasmodium* by Italian scientists, Marchiafava.
and Celli in 1889. It was a only a decade later in 1898 when the second most important discovery was made, where Sir Ronald Ross proved that the malaria parasite is transmitted via mosquitoes which in case of humans is *Anopheles spp.* A brief mention of the milestones of achievements in malaria is shown in Table 1.

**Table 1 – Few Milestones in Malaria**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Scientist Name</th>
<th>Achievements</th>
<th>Year</th>
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<tbody>
<tr>
<td>1</td>
<td>Charles Louis Alphonse Lavern</td>
<td>Discovered the malaria parasite</td>
<td>1880</td>
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<tr>
<td>2</td>
<td>Camilli Golgi</td>
<td>Two different malaria parasites responsible for tertian and quartan malaria</td>
<td>1886</td>
</tr>
<tr>
<td>3</td>
<td>Giovanni Batista Grassi and Raimondo Filetti</td>
<td>Introduced names - <em>Plasmodium vivax</em> and <em>Plasmodium malariae</em></td>
<td>1890</td>
</tr>
<tr>
<td>4</td>
<td>William H. Welch</td>
<td>Named malignant tertian malaria as <em>Plasmodium falciparum</em></td>
<td>1897</td>
</tr>
<tr>
<td>5</td>
<td>Sir Ronald Ross</td>
<td>Discovered that mosquitoes transmit malaria parasites</td>
<td>1898</td>
</tr>
<tr>
<td>6</td>
<td>Giovanni Batista Grassi</td>
<td><em>Anopheles spp</em> responsible for transmission of human malaria parasite, <em>Plasmodium</em></td>
<td>1899</td>
</tr>
<tr>
<td>7</td>
<td>John W. W. Stephens</td>
<td><em>Plasmodium ovale</em></td>
<td>1922</td>
</tr>
<tr>
<td>8</td>
<td>Hans Andersag</td>
<td>Discovery of Chloroquine</td>
<td>1934</td>
</tr>
<tr>
<td>9</td>
<td>Paul Muller</td>
<td>Discovery of the Insecticide, DDT</td>
<td>1939</td>
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</table>
1.3. The vector – *Anopheles spp.*

There are 460 species of *Anopheles* mosquito identified till date, out of which over 100 species transmit malaria. In India alone 58 anopheline species exist out of which 6 have been identified as the main vectors for malaria which are *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles fluviatilis*, *Anopheles minimus*, *Anopheles sundaicus*, *Anopheles dirus*. Some other species, i.e., *Anopheles varuna*, *Anopheles annularis s.l.*, *Anopheles philippinensis-nivipes* and *Anopheles jeyporiensis* are considered to be of local importance as secondary malaria vectors (Dash *et al.*, 2007).

The distribution pattern of the main malaria vectors of the world shown in Figure 2.
1.4. Life Cycle of *Plasmodium*

The life cycle of the parasite traverses through two hosts, human beings the intermediate host and *Anopheles* mosquito serving as the definitive host. *Plasmodium* development in *Anopheles* begins with the ingestion of the parasite by the mosquito, following which the male gametocyte undergoes three successive nuclear divisions to form eight male gametes. The gametes are liberated from the microgametocyte by the process called ex-flagellation which is believed to be induced by a drop in temperature, an increase in carbon dioxide, and mosquito metabolites like xanthurenic acids. The microgamete now fertilizes a female gamete (macrogamete, formed from the macrogametocyte) to form the zygote. Within 24 hours post-infection the zygote elongates to form ookinete which are motile invasive entities in the parasite life cycle. These motile ookinetes penetrate the midgut epithelium and
rapidly transform into immotile oocysts under the basal lamina of the midgut. After growth and multiple divisions for 10–12 days, thousands of haploid sporozoites are released from each oocyst into the hemolymph. The hemolymph circulation and sporozoite movement carry a fraction of the parasites to the salivary glands, where they are released into the saliva during subsequent blood feeding.

The asexual phase of parasite development commences in the human host post bite with the infective mosquito. The sporozoites are lodged in the skin post mosquito bite, and only a few sporozoites reach the liver via the blood vessels evading the host immune system and they glide through the blood vessels in a corkscrew movement pattern (Amino et al., 2007). The sporozoites then migrate through host cells including the liver resident Kupffer cells and hepatocytes (liver cells) before eventually invading a hepatocyte with the formation of a parasitophorous vacuole (PV) and this leads to the beginning of the liver stage growth, exo-erythrocytic stage (Frevert et al., 2005). The circumsporozoite protein (CSP) of the parasite plays a very important role in ensuring that the sporozoites reach the liver and initiate the liver development stages (Usynin et al., 2007; Singh et al., 2007). Inside the hepatocyte the sporozoites matures and develops into a schizont containing thousands of merozoites. The exo-erythrocytic stages last for 5.5-7 days in *P. falciparum*, 6-8 in *P. vivax*, 9 days in *P. ovale* and 14-16 days in *P. malariae*, before the hepatic schizonts rupture and release the merozoites that would now infect the erythrocytes. The exo-erythrocytic stage is a clinically silent phase with no symptoms and lasts a single cycle unlike the erythrocytic stage which occurs repeatedly (Vaughan et al., 2008). The merozoites developed in the hepatocytes exit the liver in host cell derived vesicles called merosomes, protecting the hepatic merozoites from phagocytosis by Kupffer cells. The merozoites are eventually released in the lung capillaries, and enter the bloodstream to commence the erythrocytic stage of parasite development (Sturm et al., 2006; Baer et al., 2007).
In case of *P. vivax* and *P. ovale*, some of the sporozoites remain dormant for months in the liver, called as hypnozoites, which after a latent period develop into schizonts resulting in relapse. It is has been suggested that the parasite strains that develop into hypnozoites are genotypically different from those that commence development in the liver after mosquito bite (Imwong et al., 2007; Chen et al., 2007).

The merozoites released from the liver invade the red blood cells (RBCs) within a matter of few seconds involving a series of receptor-ligand binding interaction on the erythrocyte surface thereby minimizing the exposure of parasite surface antigens to the host immune system (Cowman et al., 2006). The receptor-ligand interactions are mediated by various parasite proteins which include Merozoite surface proteins (MSP) (Goel et al., 2003), Apical membrane antigen-1 (AMA-1) (Mitchell et al., 2004), Duffy binding like erythrocyte binding protein (DBL-EBP) family and reticulocyte binding like protein family (RBL) (Miller et al., 2002a; Rayner et al., 2001). *P. vivax* has the ability to invade only those RBCs that have the Duffy blood group positive antigen which interacts with the Duffy binding protein (DBP) and an unknown receptor present only on the reticulocytes recognized with the help of reticulocyte binding protein (RBP) (Miller et al., 1976; Galinski et al., 1992; Kasenhagen et al., 2007). On the other hand *P. falciparum* has a highly redundant invasion pathway using several different receptor families, allowing it to invade any red cell, unlike *P. vivax* (Camus et al., 1985; Sim et al., 1994; Mayer et al., 2001; Mayer et al., 2004; Peterson et al., 2000; Curtodor et al., 2005).

Upon invasion the merozoites enter the RBCs by creating a parasitophorous vacuole (PV), seen as the intracellular ring, thereby creating a hospitable environment for its development inside the RBC (Bannister et al., 2000). Inside the RBC the parasite multiplies by firstly altering the host cell wall permeability and morphology, secondly using the host cell machinery and thirdly degrading the principle nutrient, hemoglobin in the food vacuole into amino acids and toxic heme. The
amino acids derived from hemoglobin degradation are utilized for protein biosynthesis and the toxic heme is detoxified and sequestered as hemozoin (Rudzinka et al., 1965; Rosenthal et al., 1998; Kirk, 2001). During the erythrocyte cycle the parasite traverses through the developmental stages of ring, trophozoite and schizont. At the end of each cycle the infected RBCs rupture and release fresh batch of merozoites that in turn infect more RBCs. It is during the erythrocytic cycle that the clinical symptoms appear in the patient. The erythrocytic cycle is of 48hrs in case of *P. falciparum*, *P. vivax* and *P. ovale*, and 72hrs for *P. malariae*. In *P. falciparum*, a single RBC can be infected with multiple merozoites and *P. vivax* infects young RBCs i.e. reticulocytes. During the erythrocyte stage, a subset of merozoites after infecting the fresh RBCs enter into the sexual phase by either developing into a male or female gametocyte, which when ingested by the *Anopheles* mosquito during blood meal undergo the sexual phase of development. In case of *P. falciparum* it is only the early ring stages and these sexual stages that are visible in the peripheral blood, as rest of the asexual developmental phase takes place in the capillaries. This process of sequestration of mature asexual stages is unique to *P. falciparum* and here the surface of the infected RBC (iRBC) develops small protrusions, called knobs. These knobs are the localization sites of various parasite derived proteins collectively called *pfemp1* (*Plasmodium falciparum* erythrocyte membrane protein 1) that helps them in sticking to the walls of the blood vessels known as cytoadherence as well as to uninfected RBCs (Baruch et al., 1995; Smith et al, 1995; Rowe et al., 1997). This leads to obstruction of the blood vessels which ultimately results in the dysfunction of organs including brain that leads to cerebral malaria. Thereby, *P. falciparum* is known to be one of the deadliest protozoan parasites, having a high rate of mortality if not treated in time. The life cycle of *Plasmodium* is shown in Figure 3.
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Figure 3 – Life cycle of malaria parasite, *Plasmodium*, Source – CDC, Atlanta

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release merozoites 4. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells 5. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites 6. Some parasites differentiate into sexual erythrocytic stages (gametocytes) 7. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal 8. The parasites’ multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito’s stomach, the microgametes penetrate the macrogametes generating zygotes 9. The zygotes in turn become motile and elongated (ookinetes) 10 which invade the midgut wall of the mosquito where they develop into oocysts 11. The oocysts grow, rupture, and release sporozoites 12, which make their way to the mosquito’s salivary glands. Inoculation of the sporozoites 1 into a new human host perpetuates the malaria life cycle.
1.5. The Disease- Symptoms, Diagnosis

The incubation period i.e. the time interval between the inoculation of the sporozoites in the human host and the appearance of the first clinical symptom, last for 9-14 days for *Plasmodium falciparum*, 12-17 days for *Plasmodium vivax*, 18-40 days for *Plasmodium malariae*, 16-18 days for *Plasmodium ovale*. The clinical symptoms of the disease first appear during the erythrocytic stages of the malaria parasite. The clinical symptoms include fever, headache, muscular pain, nausea, dizziness, vomiting, anaemia and splenomegaly. The classical symptom of malaria is the sudden rise and fall in body temperature, with chills and rigor after regular intervals of 48hrs to 72hrs depending on the *Plasmodium* species, but is not necessarily always seen. This rise and fall in body temperature of a malaria patient coincides with the periodic release of merozoites, ready to infect new RBCs. In addition, other symptoms include anaemia, result of the erythrocyte destruction and increased phagocytosis of infected red blood cells and splenomegaly, an enlargement of spleen caused by the accumulation of phagocytic red blood cells and dilation of sinuses. (Claire et al., 2004) Falciparum infection can result in severe disease complications like cerebral malaria, jaundice, severe anaemia, hypoglycemia, renal failure, impaired consciousness, extreme weakness, convulsions etc. These complications arise if the patient does not take treatment in time or the treatment is inadequate or the parasite is resistant to the drug being administered.

Apart from the clinical symptoms, microscopic blood examination is necessary for the confirmation of the disease. Thick and thin blood smears are examined under the microscope after staining with Giemsa or JSB stain (Singh, 1956) to confirm malaria infection with either of the *Plasmodium* species. Careful examination of the blood smear under the microscope is required for accurate identification of the infection. This approach of identification of the malaria parasite under the microscope has been for hundreds of years and still remains the gold standard for
malaria diagnosis. However, a proper expertise is necessary to give accurate diagnosis, which otherwise may lead to misdiagnosis. In addition, other methods of malaria diagnosis are available, which include most prominently species specific Rapid Diagnostic Tests (RDTs) and Polymerase Chain Reaction (PCR) assays. RDTs use the mechanism of antigen-antibody interaction. In RDTs, antigens histidine-rich protein II (HRP II) specific for *Plasmodium falciparum*, aldolase or lactate dehydrogenase (pLDH) enzymes part of the glycolytic pathway, specific for different *Plasmodium* species are utilized for parasite detection (Moody, 2002). RDT usage does not require major expertise and has made the diagnosis process faster and simpler. The other method of *Plasmodium* detection is using PCR based assays (Singh et al., 1998). This method is accurate and can detect the infection at very low parasitemia levels but has its limitations as it cannot be used in the field where on spot detection is necessary.

1.6. Malaria Control

Control of the vector population & malaria transmission using vector control strategies and killing of the malaria parasite by treatment with anti-malarials remain the main strategies to control malaria as no effective vaccine is made available till date. Vector control strategies includes indoor residual spraying (IRS) with insecticides, use of Insecticide treated mosquito nets (ITNs) and anti-larvicidal measures. Four forms of insecticides being used are organochlorines, organophosphates, carbamates and pyrethroids. IRS is the most popular and effective vector control measure being used and ITNs are being used for personal protection from mosquito bites, thereby preventing malaria transmission. Pyrethroids are the only insecticide being used for both IRS and ITNs. The various insecticides being used in India are DDT, malathion and synthetic pyrethroids. However, with the advent of insecticide resistance in the major malaria vectors, *Anopheles culicifacies* and *Anopheles stephensi* against the commonly used
insecticides, regular monitoring of the mosquito populations and use of alternative insecticides has become imperative.

On the other hand treatment of malaria patients with anti-malarials is important in order to kill the parasite. Therefore, after the confirmation of malaria in a patient, he/she is provided with proper medication following the National Drug Policy on Malaria issued by the National Vector Borne Disease Control Program (NVBDCP), Government of India. The drug policy is renewed periodically to ensure effective treatment after assessing the status of anti-malarial drug effectiveness and more importantly the status of resistance in malaria parasites against anti-malarials. In the earlier days quinine was the choice of drug for malaria treatment but soon got replaced with various new anti-malarial drugs that had less adverse side effects. Since the discovery of chloroquine in the 1940s it was widely used as an anti-malarial drug against malaria infection. Other drugs like anti-folates i.e. sulphapyrimethamine, and primaquine are also used for malaria treatment. However, with the emergence of drug resistance in *Plasmodium falciparum* against chloroquine since the 1960s and in 1973 in India (Sehgal et al., 1973) has led to the urgency in development of new anti-malarial drugs. Artemisinin is the new anti-malarial drug derived from the Chinese herb, *Artemisia annua* being used for treatment of *Plasmodium falciparum* infection. Artemisinin derivatives i.e. artesunate, artemether and arteether, are currently being used in combination with other anti-malarial compounds (Rosenthal, 2008). Combination therapy is the use two or more schizonticidal drugs with independent mode of actions, in order to improve drug efficacy and delay the development of resistant parasite as not in the case of mono drug therapy. At present malaria patients are treated following the National Drug Policy on Malaria, 2010 as shown in Table-2.
**Table 2** – Anti-Malaria Drug Treatment Schedule according to the National Drug Policy on Malaria, 2010, NVBDCP, Government of India

<table>
<thead>
<tr>
<th>P. vivax infection</th>
<th>Uncomplicated P. falciparum infection</th>
<th>Mixed infection (P. vivax + P. falciparum)</th>
<th>Severe P. falciparum infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine -25mg/kg over 3 days</td>
<td>Artesunate-4mg/kg for 3 days</td>
<td>Artesunate-4mg/kg for 3 days</td>
<td>Artesunate-2.4mg/kg (IV) at 0,12,24 hr and then once a day or</td>
</tr>
<tr>
<td>Primaquine- 0.25mg/kg for 14 days</td>
<td>Sulfadoxine (25mg/kg) + Pyrimethamine (1.25mg/kg) on day 1</td>
<td>Sulfadoxine (25mg/kg) + Pyrimethamine (1.25mg/kg) on day 1</td>
<td>Artemether- 3.2mg/kg (IM) at day 0 and then 1.6mg/kg per day or</td>
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<tr>
<td></td>
<td>Primaquine-0.75mg/kg on day 2</td>
<td>Primaquine-0.75mg/kg on day 2</td>
<td>Arteether- 150mg daily for 3 days (only for adults) or</td>
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<td></td>
<td></td>
<td></td>
<td>Quinine- 20mg/kg (IV or IM) followed by 10mg/kg every 8hrs</td>
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Chemoprophylaxis (For Travelers)

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<thead>
<tr>
<th>Short Term (upto 6 weeks)</th>
<th>Doxycycline- 100mg once daily from 2 days before travel to 4 weeks after returning from malaria endemic regions</th>
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<tbody>
<tr>
<td>Long Term (&gt; 6 weeks)</td>
<td>Mefloquine- 250mg weekly from 2 weeks before, during and 4 weeks after returning from malaria endemic regions</td>
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</table>
Besides, drug treatment loads of research work has been undertaken to develop an effective vaccine but without any success. However, recently an effective vaccine RTS, S targeting circumsporozoite protein (CSP) of the malaria parasite has shown promising results in imparting protection against *Plasmodium falciparum* in Phase III Clinical Trials (Agnandji et al., NEJM, 2011). However, there is still time before it becomes readily available to the malaria exposed populations.

### 1.7. Malaria the Infection and the counter-attack

Infection is the invasion of the body by a disease causing microorganism called as pathogens. Upon infection these pathogens invade the body cells and utilize the cell resources for their reproduction and finally may or may not destroy the infected cell. The immune system is the counter-attack machinery present in the host that limits this infection and finally eliminates the pathogen from the host body.

The exo-erythrocytic stage in malaria is a clinically silent stage but a small amount of immune response is mounted against the invading sporozoites. It has been suggested that rupture of the liver cell plasma membrane during cell traversal by the sporozoites leads to the release of cytosolic factors in the microenvironment of liver. These cytosolic factors are sensed by the Toll Like Receptor (TLR) family present on the surface of resident Kupffer cells which in turn activate NF-κB, the regulator of host inflammatory response (Torgler et al., 2008). This limits the host cell infection to some extent as it has been demonstrated that liver stages counter act the inflammation with the production of anti-inflammatory factors like heme oxygenase -1 (HO-1) (Epiphanio et al., 2008).

It is during the erythrocytic stages that the clinical symptoms appear in malaria patients. Here with the completion of every schizogony cycle at 24-72 hours depending on the *Plasmodium* species, new merozoites are
released in the bloodstream to invade more RBCs. Along with them various abnormal or foreign factors, which include malaria pigment, hemozoin, ruminants of the iRBC membrane, parasite toxins like glycophasphatidyliositol proteins (GPI) are released in the blood. The circulating monocytes in the bloodstream are the first ones to encounter these foreign/abnormal factors, and ingest them by the process of phagocytosis. The activation of monocytes commences an intracellular signaling cascade involving various adaptor proteins like MyD88, TIRAP (MAL), IRAK4, etc. Consequently this cascade leads to the production of various chemokines and inflammatory cytokines like Tumor Necrosis Factor (TNF), Interleukin -6 (IL-6), monocyte chemokine attractant protein-1 (MCP-1), macrophage inflammatory protein (MIP), IL-1, interferon-γ (IFN-γ) etc. In addition, these engulfed foreign particles are also presented on the monocyte surface with Major Histocompatibility Complex-II molecule (MHC-II) to activate the T cells that ultimately triggers the cell mediated immune response against the invading pathogen. The clinical symptoms like fever, anaemia, body-ache, vomiting, tiredness etc. are attributed to these various inflammatory cytokines and chemokines released in response to the iRBC ruminant cell membrane and other parasitic products (Clark et al., 2006).

The recognition of these pathogens by the monocytes is done with the help of Pattern Recognition Receptors (PRRs), which recognize broad structural motifs of micro-organisms, one of these pattern recognition receptors, is the Toll Like Receptor family (TLR). TLRs are present on the cell surface as well as on the surface of intracellular endocytic vacuole of the monocytes and the various antigen presenting cells (APCs) of the immune system. They are the gate-keepers for identifying the invading pathogens and activating the immune system against them.

The Toll gene and its role in protection against the invading pathogens were first identified in *Drosophila* (Lemaitre et al., 1996) and later in human (Medzhitov et al., 1997). A total of 10 TLRs till date have been identified in human. The role of TLRs in malaria was recently elucidated
as it was showed that Glycophosphatidylionsitol (GPI) of *Plasmodium falciparum* (Krishnagowda et al., 2005), host fibrinogen bound hemozoin (Barrera et al., 2011) and malaria pigment, hemozoin (Coban et al., 2005; Parroche et al., 2007) act as ligands for TLR2/TLR4 and TLR9 respectively.

It has been shown that hemozoin acts as a carrier for plasmodial DNA, which when presented to TLR-9 receptor leads to the activation of the inflammatory response (Parroche et al., 2007). This inflammatory response involves the production of various pro-inflammatory cytokines that in turn induce the production of cyclooxygenase-2 (COX-2) protein, that up regulates prostaglandins which induce fever (Ball et al., 2004). Lately, it has been shown that the parasite histone-DNA complex acts as an immunostimulatory component for TLR9 (Gowda et al., 2011).

It is believed that the disease outcome is highly dependent on the maintenance of balance between the pro-inflammatory and anti-inflammatory cytokines, and excessive production of pro-inflammatory cytokine has resulted in severity of the disease (Clark et al., 2006). Thus, this production of inflammatory cytokines is in turn dependent on the activation of the inflammatory pathway by various cell surface receptors including TLRs stimulated by the abnormal and foreign factors released from the iRBCs.

1.8. **Rationale of the study**

Till present no successful vaccine has been made available to the malaria exposed population. Moreover, in order to have an effective treatment, the genetic makeup of the parasite, *Plasmodium*, the vector, *Anopheles* and the human host plays an important role. The genetic makeup of the malaria exposed population plays a vital role, as it predisposes the individual’s immune response, mounted against the invading pathogen. Thereby, polymorphism studies of various immune genes are being carried out to understand their role in disease in
particular. These studies would firstly help in understanding the effects of gene polymorphism on the immune response. Secondly, it would also help in understanding the association of these polymorphisms with the immune response against the invading pathogen. One such molecule that has gained importance in the recent years is Toll like Receptors (TLRs). In the recent past the role of TLRs in various infectious diseases has been elucidated which include, Tuberculosis, (Ben-Ali et al., 2004) severe Sepsis, (Barber et al., 2006) Asthma, (Waltraud et al., 2004) Crohn’s disease, (Torok et al., 2004) Staphylococcal infection, (Lorenz et al., 2000) Leprosy (Bochud et al., 2003). Moreover, with the identification of non conventional ligands like glycoposphatidylionsitol (GPI) of *Plasmodium falciparum* for TLR2 (Krishnegowda et al., 2005), hemozoin bound host fibrinogen for TLR4 (Barrera et al., 2011) and parasite histone-DNA complex acts as an immunostimulatory component for TLR9 (Gowda et al., 2011), the role of TLRs in malaria caught the limelight.

A number of polymorphism studies have been carried on TLR2, TLR4, TLR9 and TIRAP (Toll-interleukin-1 receptor (TIR) domain containing adaptor protein) also known as Mal (MyD88 adaptor-like). The polymorphism at residue positions Arg677Trp and Arg753Gln in TLR2 have been associated with tuberculosis making an individual susceptible to the disease (Ben-Ali et al., 2004). Similarly, polymorphism at residue positions Asp299Gln and Thr399Ile in TLR4 have shown to confer risk for typhoid infection (Bhuvanendran et al., 2011), and risk of different types of cancers (Kutikhin et al., 2011). Nucleotide positions -T1237C and -T1486C in TLR9 have also shown to be associated with Crohn’s disease (Torok et al., 2005) and lung diseases (Pabst et al., 2011). In addition mutations at these common polymorphic positions in TLR2, 4 and 9 have been associated with malaria (Mockenhaupt et al., 2006a; Mockenhaupt et al., 2006b). Moreover, the heterozygosity at residue position Ser180Leu in TIRAP (MAL), an adaptor molecule involved in the TLR signaling cascade, has shown to impart protection against malaria.
as recently published by (Khor et al., 2007). Thus, studies regarding the allelic prevalence of TLR and TIRAP genes in various parts of the world, where malaria is an endemic disease, may provide substantial data for developing therapeutics in treatment of malaria.

India even though being one of the malaria endemic countries of the world, research on the allelic prevalence of TLR 2, 4, 9 and TIRAP in the gene pool of different malaria endemic populations has not yet been dealt with, thus making this kind of study on TLR gene polymorphism a potential area of research in malaria.

Therefore, considering it to be a prospective area of research on the Indian population residing in malaria endemic regions, this study was carried out with the following objectives in mind.

■ To study the allelic prevalence of Toll like receptor (TLR) 2 (Arg677Trp, Arg753Gln), 4 (Asp299Gly, Thr399Ile), 9 (-T1237C, -T1486C) and TIRAP (Ser180Leu) in the Indian population from malaria endemic regions.

■ To investigate whether these SNPs of TLR2,4,9 and TIRAP show any association with malaria in these malaria endemic populations of India and are under any selection pressure.

■ To study the effect of these polymorphisms on the innate immune response against exposure to *Plasmodium falciparum*.

This study would help in gathering base line data regarding the genetic makeup of individuals living in the malaria endemic regions of India. This might help in stratifying the population according to susceptibility of the disease and help in developing future therapeutics or vaccines, as developed for treatment of papillomavirus infections available as Aldara (TLR7 antagonist). In addition various therapeutic treatments are being developed for other diseases like cancer, asthma and various infectious diseases (Kanzler et al., 2007).